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[Name of item] Figure 1
[Name of item] Abstract 1
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[Type of Document] Scope of Claim(s)

[Claim 1]

A DNA amplifier in which a reaction solution containing at least a nucleic acid to be used as a template, a nucleic acid to be used as a primer, a phosphate compound, and a metal ion is caused to move through a flow channel to thereby perform the DNA amplification in the flow channel, characterized in that the flow channel comprises:

a denaturation region in which a denaturation reaction is carried out, the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template; and

a regeneration region in which an annealing reaction with a single stranded nucleic acid to be used as the template and the nucleic acid to be used as the primer and a DNA synthesis reaction by a nucleic acid synthetase are carried out, the nucleic acid synthetase immobilized in the regeneration region.

[Claim 2]

A DNA amplifier according to claim 1, wherein the denaturation region is provided with a means for heating, and wherein the regeneration region is provided with a means for cooling.

[Claim 3]

A DNA amplifier according to claim 1 or 2, wherein the nucleic acid synthetase is immobilized on beads which fill the regeneration region.

[Claim 4]

A DNA amplifier according to claim 1 or 2, wherein the nucleic acid synthetase is immobilized on an inner wall surface of the flow channel comprising the denaturation region and the regeneration region.

[Claim 5]

A DNA amplifier according to any one of claims 1 to 4, wherein the flow channel provides a plurality of the denaturation regions and the regeneration regions alternately.

[Claim 6]

A DNA amplifier according to any one of claims 1 to 5,

wherein the reaction solution comprises a first reaction solution containing at least the nucleic acid to be used as the template and a second reaction solution containing at least the nucleic acid to be used as the primer, the phosphate compound, and the metal ion,

wherein the DNA amplifier comprises a means for sending the first reaction solution to the flow channel and a means for sending the second reaction solution to the flow channel, the means for sending the first reaction solution being independent of the means for sending the second reaction solution, and

wherein the DNA amplifier comprises a flow channel through which the reaction solution having been followed through the denaturation region and the regeneration region is re-used as the first reaction solution.

[Claim 7]

A method of amplifying a DNA, the method comprising:

sending a reaction solution containing at least a nucleic

acid to be used as a template, a nucleic acid to be used as a primer, a phosphate compound, and a metal ion into a flow channel, the flow channel comprising: a denaturation region in which a denaturation reaction is carried out, the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template; and a regeneration region in which an annealing reaction with a single stranded nucleic acid to be used as the template and the nucleic acid to be used as the primer and a DNA synthesis reaction by a nucleic acid synthetase are carried out;

performing the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template in the denaturation region; and

performing the annealing reaction with the single stranded nucleic acid to be used as the template and the nucleic acid to be used as the primer in the regeneration region and the DNA synthesis reaction by the nucleic acid synthetase immobilized in the regeneration region.

[Type of Document] SPECIFICATION

[Title of the Invention] DNA Amplifier and Method of DNA amplification

[Technical Field]

[0001]

The present invention relates to a DNA amplifier and a method of DNA amplification which utilizes a PCR method. More specifically, the present invention relates to a DNA amplifier in which a reaction solution containing at least a nucleic acid to be used as a template, a nucleic acid to be used as a primer, a phosphate compound, and a metal ion is caused to move through a flow channel to thereby carry out the DNA amplification by the DNA synthetase immobilized in the flow channel, and to a method of DNA amplification performed therewith.

[Background Art]

[0002]

For efficient replication and amplification of a minute amount of template DNA, a polymerase chain reaction (PCR) method has been widely used. The PCR method is a method of amplifying a target DNA involving one cycle of the steps of: forming single-stranded DNAs by thermal denaturation of a double-stranded DNA provided as a template; annealing each of the obtained single-stranded DNAs with its complementary primer; and synthesizing a double-stranded DNA by forming a complementary strand from the primer by the action of a heat-resisting DNA polymerase, the cycle being repeated two or more times.

[0003]

Each of the above steps is carried out with managements on the temperature of a reaction solution and reaction time. Generally, the thermal denaturation of a double-stranded DNA provided as a template to single-stranded DNAs is carried out at about 94°C, the annealing of a primer to each of the single-stranded DNAs is carried out at about 55°C, and the synthesis of a complementary strand with a DNA polymerase is carried out at about 72°C.

[0004]

Conventionally, a device which has been known in the art as a device that performs a PCR method automatically is of placing a reaction solution containing template DNA, primers, dNTPs, DNA polymerase, and the like in an Eppendorf tube and then inserting the tubes in the respective wells formed in an aluminum block to carry out reactions by changing the temperature of the block using a heater and a cooler.

[0005]

However, the PCR method requires heat cycles be carried out under accurate temperature-controls. Any reaction in a batch system like the one described above has been limited in scale-up because thermal fluctuation in a reaction system increases extensively as the scale of the reaction increases.

[0006]

Therefore, as a PCR method capable of carrying out the temperature-control of the heat cycles with high accuracy and also permitting scale-up, a flow type PCR method is disclosed in Patent Document 1 and Non-Patent Document 1 listed below.

This flow type PCR method is a method involving carrying out heat cycles by introducing a reaction solution containing DNA polymerase, template DNA, primer DNAs, dNTPs, and so on into a flow channel having a heating portion and a cooling portion.

[0007]

In addition, in Patent Document 2 listed below, there is disclosed a method of nucleic acid sequence amplification, characterized by including the steps of: (a) synthesizing a primer-elongation chain complimentary to a template by treating a nucleic acid to be used as the template with at least one primer substantially complimentary to the base sequence of the nucleic acid and a DNA polymerase, in which the primer is a chimera oligonucleotide primer containing deoxyribonucleotides and ribonucleotides, the ribonucleotides being arranged on the 3' end or 3' direction thereof for being cleaved by an endonuclease; (b) cleaving a ribonucleotide-containing moiety of the primer-elongation chain of a double-stranded nucleic acid obtained in the step (a) by means of endonuclease; and (c) carrying out chain substitution by elongating a nucleic acid sequence complementary to the template by means of a DNA polymerase having chain-substitution activity from the 3' end of the primer portion of the double-stranded nucleic acid obtained in the step (b), from which the primer-elongation chain is cleaved. According to this method (ICAN method), DNA can be amplified without any heat cycle, so that an enzyme having no heat resistance property can be used and a reaction scale is not restricted by thermal fluctuation.

Patent Document 1: JP 06-30776 A

Patent Document 2: JP 2003-70490 A

Non-Patent Document 1: "Science" (1998) 280 5366, p.1046-1048 (Written by Kopp MU, Mello AJ, and Manz A.)

[Disclosure of the Invention]

[Problems that the Invention is to Solve]

[0008]

However, any of the above batch type PCR method and the PCR methods described in Patent Document 1 and Non-Patent Document 1 requires heating in denaturation of a double-stranded template DNA to single-stranded DNAs, and therefore a specific DNA polymerase having heat resistance property is required. Therefore, there is a disadvantage in that none of the DNA polymerases having no heat resistance property, which generally exist in nature, can be used.

[0009]

In addition, the method disclosed in the above Patent Document 2 employs a chimera primer composed of RNA and DNA as a primer, or requires a specific enzyme such as exo-Bca DNA polymerase that synthesizes DNA while winding off the double strand of DNA and RNase H which cleaves the contact point between DNA additionally elongated from the chimera primer and a chimera primer RNA. Thus, there is a disadvantage of increasing cost.

[0010]

Furthermore, in the above conventional methods, the reaction product is contaminated with nucleic acid synthetases such as DNA polymerase. Thus, the purification of amplified DNA will

take much time and almost no recycle of expensive nucleic acid synthetases was possible.

[0011]

Therefore, an object of the present invention is to provide a nucleic acid amplifier by which PCR can be continuously performed in an efficient manner not only in the case of using a nucleic acid synthetase having heat resistance property but also in the case of using one having no heat resistance property, the nucleic acid synthetase can be recycled and continuously utilized, and also the reaction can be scaled up while the isolation and purification of an amplified nucleic acid are facilitated, and a method of nucleic acid amplification performed therewith.

[Means of Solving the Problems]

[0012]

In order to achieve the objects, the DNA amplifier of the present invention is a DNA amplifier in which a reaction solution containing at least a nucleic acid to be used as a template, a nucleic acid to be used as a primer, a phosphate compound, and a metal ion is caused to move through a flow channel to thereby perform the DNA amplification in the flow channel, characterized in that the flow channel comprises: a denaturation region in which a denaturation reaction is carried out, the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template; and a regeneration region in which an annealing reaction with a single stranded nucleic acid to be used as the template and the nucleic acid to be used

as the primer and a DNA synthesis reaction by a nucleic acid synthetase are carried out, the nucleic acid synthetase immobilized in the regeneration region.

[0013]

According to the DNA amplifier of the present invention, when a reaction solution containing at least a nucleic acid to be used as a template, a nucleic acid to be used as a primer, a phosphate compound, and a metal ion is caused to move through one flow channel comprising: a denaturation region in which a denaturation reaction is carried out, the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template; and a regeneration region in which an annealing reaction with a single stranded nucleic acid to be used as the template and the nucleic acid to be used as the primer and a DNA synthesis reaction by a nucleic acid synthetase immobilized in the flow channel are carried out, the nucleic acid synthetase is not influenced by heating or the like in denaturing the nucleic acid to be used as a template into single strands. Thus, the nucleic acid synthetase is prevented from deactivation, so that PCR can be carried out continuously even if any nucleic acid synthetase having no heat resistance property is used. In addition, as the nucleic acid synthetase is being immobilized, the isolation and purification of an amplified DNA can be easily carried out. Besides, the nucleic acid synthetase can be recycled and continuously utilized, and the scale-up of the reaction can be also facilitated. Here, in the present invention, the term "nucleic acid" means any of nucleic acids

that include those of both natural and non-natural types.

[0014]

In the DNA amplifier of the present invention, it is preferable that the denaturation region is provided with a means for heating and that the regeneration region is provided with a means for cooling.

[0015]

According to this aspect of the present invention, a series of PCR cycles can be continuously carried out in an efficient manner, in which each cycle includes the steps of: thermally denaturing a nucleic acid to be used as a template into single strands; annealing obtained single stranded nucleic acids to be used as the template with complementary primers thereto; and synthesizing complementary strands from the primers by reacting with a nucleic acid synthetase.

[0016]

The nucleic acid synthetase is preferably immobilized on beads which fill the regeneration region.

[0017]

According to this aspect of the present invention, the immobilized nucleic acid synthetase can be efficiently contacted with a reaction solution, to thereby increase the reaction efficiency.

[0018]

The nucleic acid synthetase may be immobilized on the inner wall surface of the flow channel comprising the denaturation region and the regeneration region.

[0019]

According to this aspect of the present invention, the flow channel on which the nucleic acid synthetase is immobilized can be easily formed.

[0020]

Furthermore, a plurality of the denaturation region and the regeneration region are provided alternately in the flow channel.

[0021]

According to this aspect of the present invention, two or more PCR cycles are carried out and thus the target nucleic acid can be efficiently amplified.

[0022]

It is preferable that the reaction solution comprises a first reaction solution containing at least the nucleic acid to be used as the template and a second reaction solution containing at least the nucleic acid to be used as the primer, the phosphate compound, and the metal ion, that the DNA amplifier comprises a means for sending the first reaction solution to the flow channel and a means for sending the second reaction solution to the flow channel, the means for sending the first reaction solution being independent of the means for sending the second reaction solution, and that the DNA amplifier comprises a flow channel through which the reaction solution having been followed through the denaturation region and the regeneration region is re-used as the first reaction solution.

[0023]

According to this aspect of the present invention, the

template can be prevented from depletion and the nucleic acid to be used as the template, the nucleic acid to be used as the primer, the phosphate compound, and the like can be recycled positively, so that running costs can be reduced.

[0024]

The method of amplifying a DNA of the present invention comprising: sending a reaction solution containing at least a nucleic acid to be used as a template, a nucleic acid to be used as a primer, a phosphate compound, and a metal ion into a flow channel, the flow channel comprising: a denaturation region in which a denaturation reaction is carried out, the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template; and a regeneration region in which an annealing reaction with a single stranded nucleic acid to be used as the template and the nucleic acid to be used as the primer and a DNA synthesis reaction by a nucleic acid synthetase are carried out; performing the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template in the denaturation region; and performing the annealing reaction with the single stranded nucleic acid to be used as the template and the nucleic acid to be used as the primer in the regeneration region and the DNA synthesis reaction by the nucleic acid synthetase immobilized in the regeneration region.

[0025]

[Effects of the Invention]

According to the present invention, when a reaction solution

containing at least a nucleic acid to be used as a template, a nucleic acid to be used as a primer, a phosphate compound, and a metal ion is caused to move through one flow channel comprising: a denaturation region in which a denaturation reaction is carried out, the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template; and a regeneration region in which an annealing reaction with a single stranded nucleic acid to be used as the template and the nucleic acid to be used as the primer and a DNA synthesis reaction by a nucleic acid synthetase immobilized in the flow channel are carried out, the nucleic acid synthetase is not influenced by heating or the like in denaturing the nucleic acid to be used as a template into single strands. Thus, the nucleic acid synthetase is prevented from deactivation, so that PCR can be carried out continuously even if any nucleic acid synthetase having no heat resistance property is used. In addition, as the nucleic acid synthetase is being immobilized, the isolation and purification of an amplified DNA can be easily carried out. Besides, the nucleic acid synthetase can be recycled and continuously utilized, and the scale-up of the reaction can be also facilitated.

[Best Mode for carrying out the Invention]

[0026]

At first, the method of DNA amplification of the present invention will be described.

The method of DNA amplification of the present invention involves introducing a reaction solution containing at least

a nucleic acid to be used as a template (hereinafter, simply referred to as a template), a nucleic acid to be used as a primer (hereinafter, simply referred to as a primer), a phosphate compound, and a metal ion into one flow channel to denature the template in the flow channel, executing annealing between the denatured template and the primer, and synthesize a DNA with a nucleic acid synthetase. The flow channel is constructed of: a denaturation region for carrying out a denaturation reaction of the double-stranded nucleic acid to be used as a template; and a regeneration region for carrying out an annealing reaction between the single-stranded nucleic acid to be used as a template and the primer and also carrying out a nucleic acid synthesis reaction with a nucleic acid synthetase, where the nucleic acid synthetase is immobilized on at least part of the regeneration of the flow channel.

[0027]

The denaturation region is set to an environment required for the denaturation of a template, for instance, set to any of environmental conditions of (1) being adjusted to the melting temperature of the nucleic acid or higher, (2) being adjusted to acidic or basic, (3) containing no cation, or (4) being mixed with a hydrogen-bond inhibitor (e.g., urea or guanidium salt). Here, the term "denaturation of a template" means that a double-stranded nucleic acid is melted and converted to single-stranded nucleic acids.

[0028]

In the present invention, as the denaturation and

regeneration of a nucleic acid are repetitively carried out, among the above conditions, it is preferable to set to one being adjusted to the melting temperature of the nucleic acid or higher (heating is more effective as means) or being adjusted to acidic or basic because it can be set repetitively. Particularly preferable is to adjust to the melting temperature of the nucleic acid or higher because it is most effective. For instance, the template is denaturated by heating at a temperature equal to the melting temperature of the nucleic acid or higher, the template may be heated at 90 to 99°C, preferably 92 to 97°C, when the template has a length of several hundred mer although the temperature varies from case by case depending on the length or arrangement of the template.

[0029]

Here, it is difficult to impart resistance to bases to a nucleic acid synthetase. In the conventional PCR method, therefore, no basic environment has been used as a template denaturation condition. In the present invention, however, the region on which the nucleic acid synthetase is immobilized may be set to a neutral environment. Therefore, the denaturation region can be set to a basic environment so that the template denaturation is carried out.

[0030]

On the other hand, the regeneration region is set to an environment required for the DNA regeneration, for example, any of the environment that satisfies all of conditions of (1) being adjusted to the dissociation temperature of a nucleic acid or

lower (by means of non-heating or cooling), (2) being adjusted to be a mild acid or mild base (approximately pH 7 ± 3), (3) containing appropriate cations, and (4) containing no hydrogen-bond inhibitor (e.g., urea or guanidium salt). For instance, the temperature for carrying out the DNA regeneration, which varies from case by case depending on the dessociation temperature depending on the template and the primer, may be, for example, 30 to 70°C when the primer of 15 to 30 mer is used. In the present invention, the temperature is particularly preferably 30 to 40°C. Here, the term "DNA regeneration" means the formation of a double strand between single-stranded DNA complementary to each other, or between RNA and DNA. Thus, the DNA regeneration under the environment for carrying out PCR substantially means annealing between the template and the primer.

[0031]

In the present invention, for heating the reaction solution moving in the flow channel to the dissociation temperature of the nucleic acid or higher, the denaturation region is preferably formed by providing with a means for heating outside the flow channel. On the other hand, for adjusting the reaction solution moving in the flow channel to the dissociation temperature of the nucleic acid or lower, the regeneration region is preferably formed by providing with a means for cooling outside the flow channel.

[0032]

The nucleic acid synthetase used in the method of the present

invention is an enzyme which can be used for the nucleic acid amplification, and is not specifically limited as far as it is any of those which are commonly available. Concrete examples of the enzymes include DNA polymerase, ligase, and reverse transcriptase. In addition, RNA polymerase may be used in combination with the nucleic acid synthetase.

[0033]

Here, in the present invention, a nucleic acid synthetase having heat resistance property, which has been used in the conventional PCR or ligase chain reaction (LCR) method, may be used. In addition, as the nucleic acid synthetase is immobilized on the flow channel of the regeneration region without being exposed to heat or the like generated in the template denaturation, any nucleic acid synthetase having no heat resistance property can be used.

[0034]

In the present invention, an enzyme having high reaction efficiency or an enzyme easily obtainable can be preferably used. Concretely, DNA polymerase I derived from *Escherichia coli*, which shows high fidelity in replication, is preferably used. In addition, a Klenow fragment or the like prepared by removing an exonuclease active site from the DNA polymerase I may be used though fidelity in replication slightly reduces.

[0035]

The nucleic acid synthetase may be, for example, immobilized on the surface of beads and then filled in at least part of the regeneration region of the flow channel, or may be directly

immobilized on the inner wall surface of the flow channel.

[0036]

Examples of the material of beads for immobilizing the nucleic acid synthetase may preferably include, but not specifically limited to, metal fine particles, glass particles, and resin particles. In particular, beads having good affinity to a biomolecule and capable of immobilizing an enzyme thereon easily, such as latex beads, are preferably used. The size of each of the beads may be any size enough to be filled in the flow channel and may be suitably defined, but is generally 0.4 to 100 μm, preferably 1 to 50 μm in diameter.

[0037]

In addition, the flow channel may be preferably formed of a material having comparatively high heat conductivity, stability in the temperature range required for PCR, resistance to erosion with an electrolytic solution or an organic solvent, and difficulty in adsorption of nucleic acid or protein. Examples of materials having heat resistance property and corrosion resistance include glass, quartz, silicon, and various kinds of plastics. Furthermore, it is preferable that the surface of any of those materials (the inner wall surface to be in contact with the reaction solution) be coated with a material, such as polyethylene and polypropylene, generally known to be difficult in adsorption of nucleic acid or protein. Alternatively, it is preferable to prevent the adsorption of nucleic acid or protein by introduction of any molecule rich in hydrophilic functional groups, such as polyethylene glycol

(PEG), via a covalent bond or the like.

[0038]

Any of well-known methods including a supporting or inclusion method, a covalent-binding method, a cross-linking method, and an electrostatic adsorption method may be adopted as a method of immobilizing the nucleic acid synthetase on the surface of the beads or the inner wall surface of the flow channel. For repeating the enzyme reaction, among them, particularly preferable is the covalent-binding method or cross-linking method. For instance, the covalent-binding method can be performed on the basis of the method described in JP-A-3-164177. A comparatively highly reactive functional group (e.g., a chlorocarbonyl group (carboxylate chloride), a carboxyl group, an amino group, a thiol group (sulfanyl group), or an epoxy group) may be introduced into the surface of beads or the inner wall surface of the flow channel to allow such a functional group to react with a carbonyl group, an amino group, or a thiol group (sulfanyl group) on the surface of the nucleic acid synthetase, thereby attaining the immobilization.

[0039]

The reaction solution used in the present invention may contain at least a template, a primer, a phosphate compound, and a metal ion.

[0040]

The template described above is a nucleic acid, an amplification target, which may be any of natural or non-natural type nucleic acids prepared by the conventional method. The

concentration of the template in the reaction solution is, in general, preferably 0.01 to 100 pM, more preferably 0.1 to 10 pM.

[0041]

The primer is a nucleic acid having a base sequence complementary to at least part of the base sequence of the template and may be any of those used in the common PCR or LCR method. However, it is preferable to design such a primer so as to efficiently amplify the target nucleic acid, and one having a length of 15 to 30 mer is generally preferably used. For instance, the DNA to be used as a primer may be one easily prepared using an automated DNA synthesizer. The concentration of the primer in the reaction solution is, in general, preferably 0.01 to 1 μM, more preferably 0.1 to 0.2 μM. Furthermore, the primers described above include chemically modified or altered non-natural type nucleic acids for a subsequent detection or isolation process. Preferable examples of the above non-natural type nucleic acids include, but not specifically limited to, oligonucleic acids labeled with biotin or FITC, oligonucleic acids having phosphotioate bindings, and chimeric nucleic acid containing peptide nucleic acid (PNA) and natural type nucleic acid.

[0042]

The phosphate compound is a component to be provided as a substrate for the amplification of nucleic acid. For instance, when a DNA polymerase or a reverse transcriptase is used as a nucleic acid synthetase, a mixture containing dNTPs (i.e., dATPs,

dCTPs, dGTPs, and dTTPs) at any ratio, preferably four kinds of deoxynucleotide triphosphate at equal ratio may be used. On the other hand, when ligase is used, it is preferable to use NTP, and ATP or GTP can be particularly preferably exemplified. The concentration of the phosphate compound in the reaction solution can be suitably defined. In general, however, it is preferably 0.01 to 1 mM, more preferably 0.1 to 0.5 mM.

[0043]

For the metal ion, a potassium ion (K^+), a sodium ion (Na^+), or a magnesium ion (Mg^{2+}) may be exemplified. Including such a metal ion makes it possible to attain effects on improvements in stability of double-stranded DNA, enzyme activity, and faithfulness of synthesized DNA. In general, the concentration of the metal ion in the reaction solution is preferably 10 to 200 mM, more preferably 50 to 100 mM for the potassium or sodium ion. Alternatively, for the magnesium ion, the concentration is preferably 1 to 5 mM, more preferably 1.5 to 2.5 mM.

[0044]

In the method of the present invention, for effectively carrying out the denaturation of a template, the annealing between the denatured template and the primer, and the synthesis of DNA in the flow channel, it is preferable that condition such as the rate of sending the reaction solution and the length of flow channel be suitably adjusted. Those conditions may vary from case by case depending on the length of the template, the length of the DNA to be synthesized, the reaction rate with the nucleic acid synthetase used, or the like. In general, however,

the time period required for the reaction solution to pass through the denaturation region once is 1 to 60 seconds, preferably 5 to 30 seconds, and also the time period required for the solution to pass through the regeneration region once is 5 to 300 seconds, preferably 10 to 120 seconds.

[0045]

Hereinafter, the DNA amplifier used in the method of DNA amplification of the present invention will be described with reference to the attached drawings, but basically the same parts are provided with the same reference numerals or signs to omit the explanations thereof.

[0046]

Fig. 1 illustrates one of the embodiments of the DNA amplifier of the present invention. A DNA amplifier 10 includes: a base plate 1 having a heating region and a cooling region; and a flow channel 2 formed on the base plate, the flow channel 2 having a predetermined inner diameter and passing through both the heating region and the cooling region two or more times while snaking its way alternately in the heating region and the cooling region. Part of the regeneration region of the flow channel is provided with plural beads-filling parts 3 in which beads having the nucleic acid synthetase immobilized on the surface thereof. In addition, both sides of the flow channel 2 are provided with an injection pore 2a for injecting the reaction solution into the flow channel and a discharge pore 2b for discharging the reaction solution after completion of the amplification reaction of DNA.

[0047]

Furthermore, Fig. 2 is an enlarged schematic diagram of a part of the flow channel of the DNA amplifier. The beads-filling part 3 is filled with an immobilized nucleic-acid synthesizing enzyme 6, which is prepared by immobilizing a nucleic acid synthetase 5 on the surface of beads 4, such that a reaction solution which has moved along the flow channel can contact with the nucleic acid synthetase 5. This beads-filling part 3 may be formed immediately downstream of the cooling region. Furthermore, when the immobilized nucleic-acid synthesizing enzyme 6 is filled in the flow channel, for preventing the leakage of the immobilized nucleic-acid synthesizing enzyme 6, it is preferable to install a filter having an appropriate filtration size downstream of the beads-filling part 3. Examples of a material of the filter preferably include, but not specifically limited to, one on which any nucleic acid is hardly absorbed, such as cellulose.

[0048]

According to the DNA amplifier 10, by an external solution-sending device (not shown) such as a pump, a reaction solution containing at least a template, a primer, a phosphate compound, and a metal ion is fed in the flow channel 2 in the direction along the arrow shown in the figure. Consequently, after the template has been converted into single strands in the heating region by means of thermal denaturation, the single-stranded template is subjected to an annealing reaction with a primer complementary to the template in the cooling region.

Furthermore, a complementary strand with respect to the single-stranded template is synthesized by means of the immobilized nucleic-acid synthesizing enzyme 6 in the beads-filling part 3. Therefore, one cycle of PCR is carried out every time the reaction solution passes through both of the heating and cooling regions.

[0049]

In the present invention, the flow channel 2 may be formed such that the flow channel passes each of the heating region and the cooling region once or more. For efficiently carrying out the DNA amplification, it is preferable to make the flow channel so as to pass through each of them 20 to 40 times.

[0050]

In addition, the size of the flow channel 2 is preferably defined such that thermal fluctuation can be prevented through facilitating heat conduction by extending a specific surface area while reducing the diameter of the flow channel 2 (see Science No. 280, vol. 5366, pages 1046-1048 (written by Kopp MU, Mello AJ, and Manz A), 1998). Specifically, the optimal width of the flow channel is 20 to 200 μm, preferably 50 to 100 μm, and the optimal depth thereof is 20 to 200 μm, preferably 40 to 100 μm. Furthermore, the width of the flow channel corresponding to the portion to be filled with the immobilized nucleic-acid synthesizing enzyme 6 is 20 to 3,000 μm, preferably 50 to 1,000 μm, and the depth thereof is 20 to 1,000 μm, preferably 40 to 500 μm.

[0051]

In addition, the flow channel 2 may be preferably formed of a material having comparatively high heat conductivity, stability in the temperature range required for PCR, resistance to erosion with an electrolytic solution or an organic solvent, and difficulty in adsorption of nucleic acid or protein. Examples of materials having heat resistance property and corrosion resistance include glass, quartz, silicon, and various kinds of plastics. Furthermore, it is preferable that the surface of any of those materials (the surface to be in contact with the reaction solution) be coated with a material, such as polyethylene and polypropylene, generally known to be difficult in adsorption of nucleic acid or protein. Alternatively, it is preferable to prevent the adsorption of nucleic acid or protein by introduction of any molecule rich in hydrophilic functional groups, such as polyethylene glycol (PEG), via a covalent bond or the like.

[0052]

The base plate having the flow channel can be, for example, formed as follows. That is, a process may involve: forming, on a single base plate made of the above material, a groove having the predetermined width and depth as defined above by cutting work or the like; and attaching another base plate or a film so as to cover the groove.

[0053]

Fig. 3 illustrates another embodiment of the DNA amplifier of the present invention. This DNA amplifier 20 is designed such that the base plate 1 shown in Fig. 1 is connected to a

plurality of other base plates in a branched configuration. The connections of those base plates are not limited to the configuration shown in Fig. 3. Any of various configurations may be chosen for performing efficient nucleic acid amplification.

[0054]

The DNA amplifier 20 employs a reaction solution consisting of a first reaction solution containing at least the above template and a second reaction solution containing at least the above primer, the phosphate compound, and the metal ion. At first, by means of an external solution-sending device 11 such as a pump, the first reaction solution and the second reaction solution are supplied to the base plate 1a from the first reaction solution chamber 14 and the second reaction solution chamber 15, respectively. Then, the reaction solution having passed through the base plate 1a is supplied directly by means of the external solution-sending device 12 as being a template to the base plates 1b to 1g. For refilling reaction substrates such as the primer and the phosphate compound which have been consumed in the reaction at the base plate 1a, it is also configured that the second reaction solution can be supplied.

[0055]

Then, the reaction solution having passed through the base plates 1b to 1f may be directly recovered and the DNA may be then purified. Alternatively, a plurality of additional base plates may be connected if required to carry out the amplification of DNA.

[0056]

Here, in this embodiment, flow channels 7 and 8 and a pump 13 are provided, thereby recycling part of the reaction solution having passed through the base plate 1a and one having passed through the base plate 1g, the reaction solution being recycled as the first reaction solution. By making such a recycling flow channel, the template can be prevented from depletion, so that the continuous amplification of nucleic acid can be stably carried out, thereby allowing reductions in running costs.

[0057]

Furthermore, when the base plates are connected to each other in a branched configuration, it is preferable that a nucleic acid synthetase having high fidelity of replication be immobilized on at least one base plate on each stage, for example, a base plate (base plate 1a) just before branching and a base plate (base plate 1g) having a channel connected for recycling a reaction solution having passed through the base plate as a first reaction solution. Consequently, the template amplification can be performed precisely, so that the template can be precisely amplified even if PCR is carried out repetitively.

[0058]

In the DNA amplifier of the present invention, the heating region and the cooling region of can be formed, for example as shown in Fig. 4(a), by installing a base plate 1 into a temperature-controlling device 33 having a structure in which a thermostatic chamber 31 having a means for heating 34 and a

thermostatic chamber 32 having a means for cooling 35 are partitioned by a partition plate 38. Furthermore, the thermostatic chambers are each provided with a stirrer 36 or 37 in order to stir media in the thermostatic chambers and to keep the temperature uniform.

[0059]

In addition, as shown in Fig. 4(b), two or more base plates 1 are laminated. Then, the means for heating 39 and the means for cooling 40 may be arranged between the adjacent base plates or between the base plates every several plates to form the heating region and cooling region of the base plate.

[0060]

Here, the means for heating and the means for cooling may be kept at predetermined temperatures by means of any temperature-controlling device. Concretely, preferable example may be a thermoelectric device, a thermostat, or the like.

[0061]

Fig. 5 illustrates still another embodiment of the DNA amplifier of the present invention. The DNA amplifier 50 uses two capillaries 51 as flow channels. The capillaries 51 are placed in the temperature-controlling device 52 having the heating region and the cooling region such that the capillaries 51 spiral so as to pass alternately through the heating region and the cooling region.

[0062]

On the inner wall surfaces of the capillaries 51, as shown

in Fig. 6, nucleic acid synthetases 4 are directly immobilized.

[0063]

The capillaries may be preferably made of, but not specifically limited to, a material having comparatively high heat conductivity, stability in the temperature range required for PCR, resistance to erosion with an electrolytic solution or an organic solvent, and hardly adsorbing nucleic acid or protein. For example, glass and plastics can be exemplified. Furthermore, it is preferable that the surface of any of those materials (the surface to be in contact with the reaction solution) be coated with a material, such as polyethylene and polypropylene, which is generally known to hardly adsorbing nucleic acid or protein. Alternatively, it is preferable to prevent the adsorption of nucleic acid or protein by introduction of any molecule rich in hydrophilic functional groups, such as polyethylene glycol (PEG), via a covalent bond or the like.

[0064]

In addition, any capillary made of a material having the property of semi-permeability that permeates only a low molecular weight substance without passing a high polymer molecule. In this case, a medium of the thermostatic chamber on which such a capillary is mounted may be a solution containing a substrate of a low molecular weight (e.g., dNTP or NTP) to supply a reaction substrate continuously into the capillary. The semi-permeable capillary may be preferably exemplified by hollow fiber available from Mitsubishi Rayon Co., Ltd., Toray Industries. Inc., or the like.

[0065]

In the present invention, the capillary has an outer diameter of 100 to 1,000 μm, preferably 200 to 500 μm, and an inner diameter of 20 to 600 μm, preferably 50 to 150 μm.

[0066]

The immobilization of the nucleic acid synthetase on the inner wall of the capillary can be performed by the same method as that of immobilizing the nucleic acid synthetase described above. The nucleic acid synthetase may be immobilized on the entire inner wall surface of the capillary. According to this, efforts of loading beads and immobilizing the nucleic acid synthetase only on a specific portion can be saved. Hear, when the nucleic acid synthetase is immobilized on the whole inner wall surface of the capillary, the nucleic acid synthetase immobilized on the heating region may be deactivated by heating or the like. But, there is no problem as long as the nucleic acid synthetase immobilized on the regeneration region has activity.

[Industrial Applicability]

[0067]

The present invention is applicable to efficiently replicate and amplify a minute amount of template DNA according to the PCR method.

[Brief Explanation of the Drawings]

[0068]

[Fig. 1] A diagram that illustrates an embodiment of the DNA amplifier of the present invention.

[Fig. 2] A schematic diagram of part of a flow channel of the DNA amplifier shown in Fig. 1.

[Fig. 3] A diagram that illustrates another embodiment of the nucleic acid amplifier of the present invention.

[Fig. 4] An explanatory diagram of a means for heating and a means for cooling for forming a heating region and a cooling region, respectively.

[Fig. 5] A diagram that illustrates still another embodiment of the DNA amplifier of the present invention.

[Fig. 6] A schematic diagram that illustrates a nucleic acid synthetase immobilized in a capillary.

[Description of Symbols]

[0069]

1, 1a to 1g base plates

2 flow channel

2a injection pore

2b discharge pore

3 beads-filling part

4 beads

5 nucleic acid synthetase

6 immobilized nucleic-acid synthesizing enzyme

10, 20, 50 DNA amplifier

11 to 13 external solution-sending device

31, 32 thermostatic chamber

33, 52 temperature-controlling device

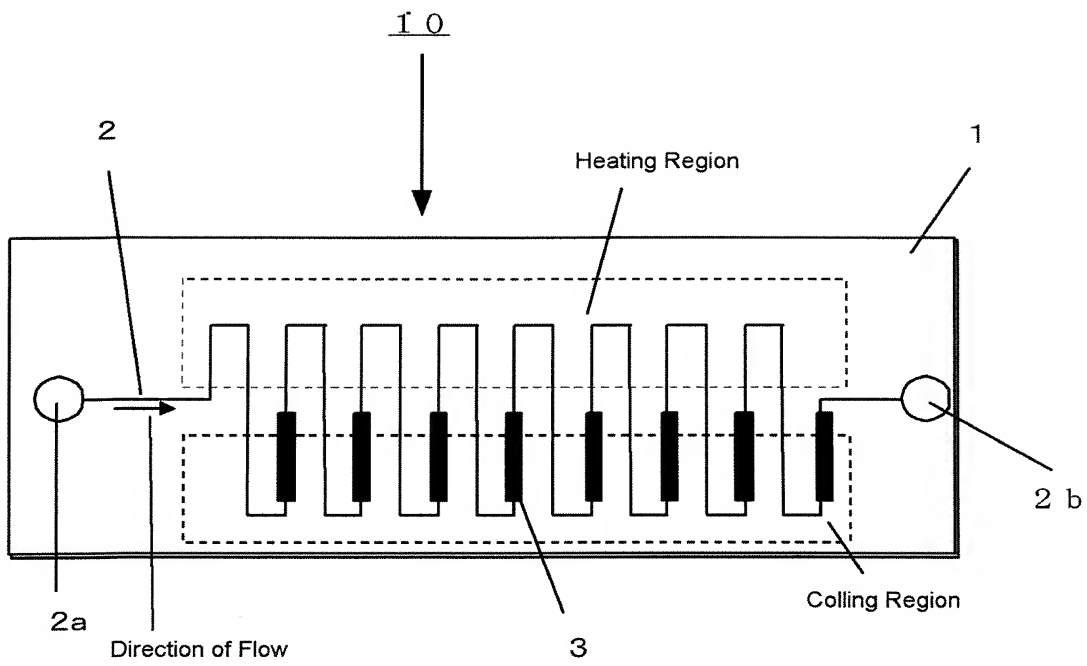
34, 39 means for heating

35, 40 means for cooling

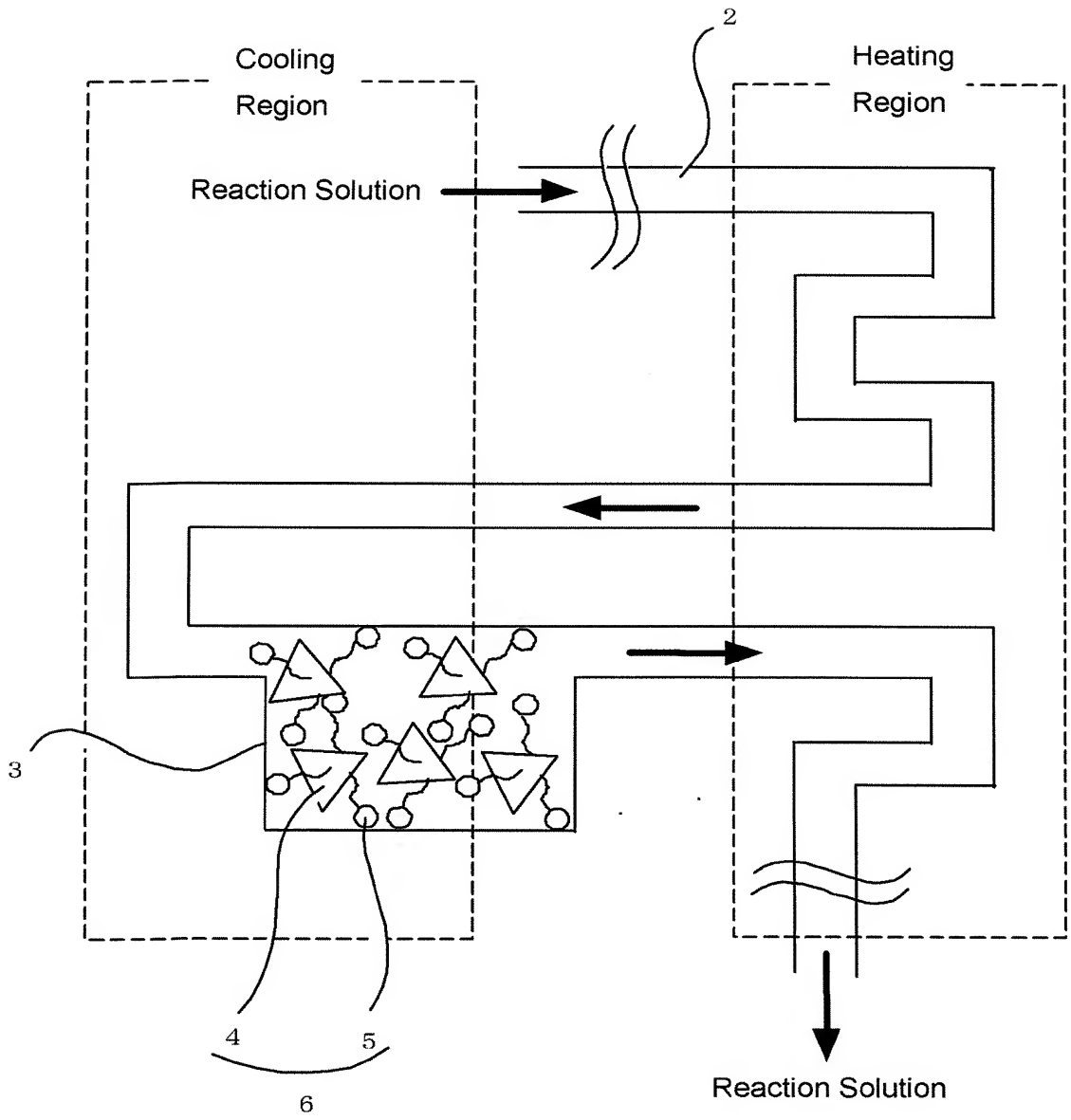
51 capillary

[Type of Document] FIGURE

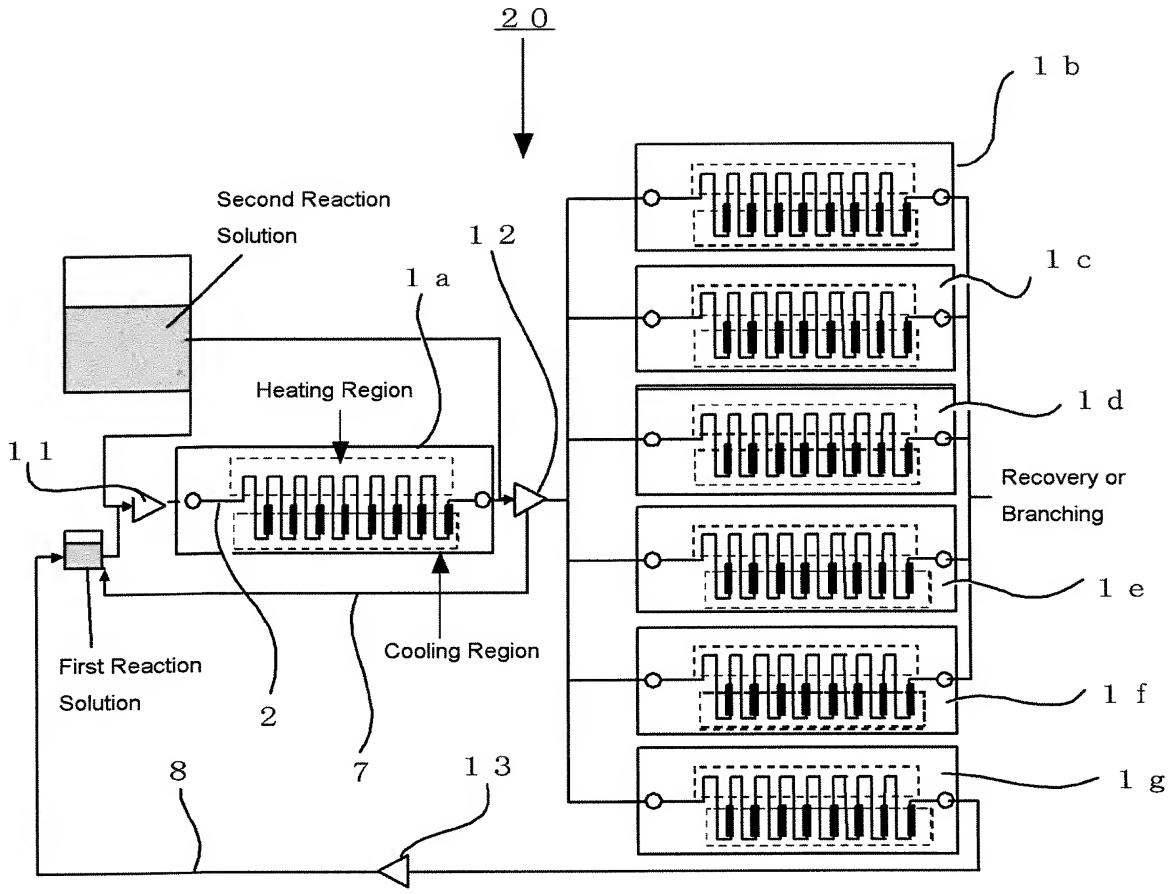
[Fig. 1]



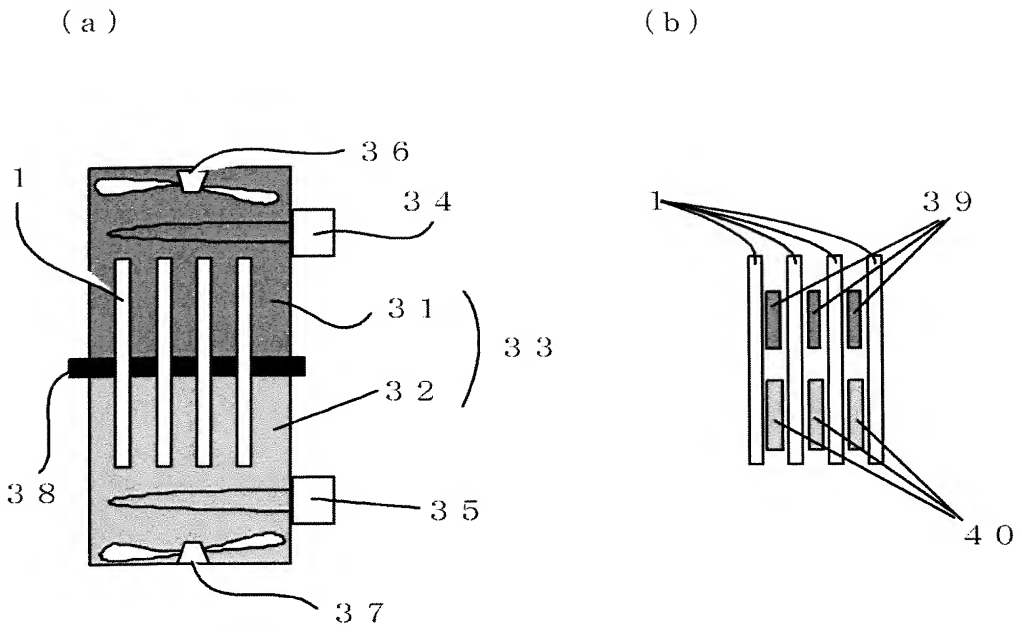
[Fig. 2]


2
Cooling
Region
Heating
Region
Reaction Solution
3
4
5
6
Reaction Solution

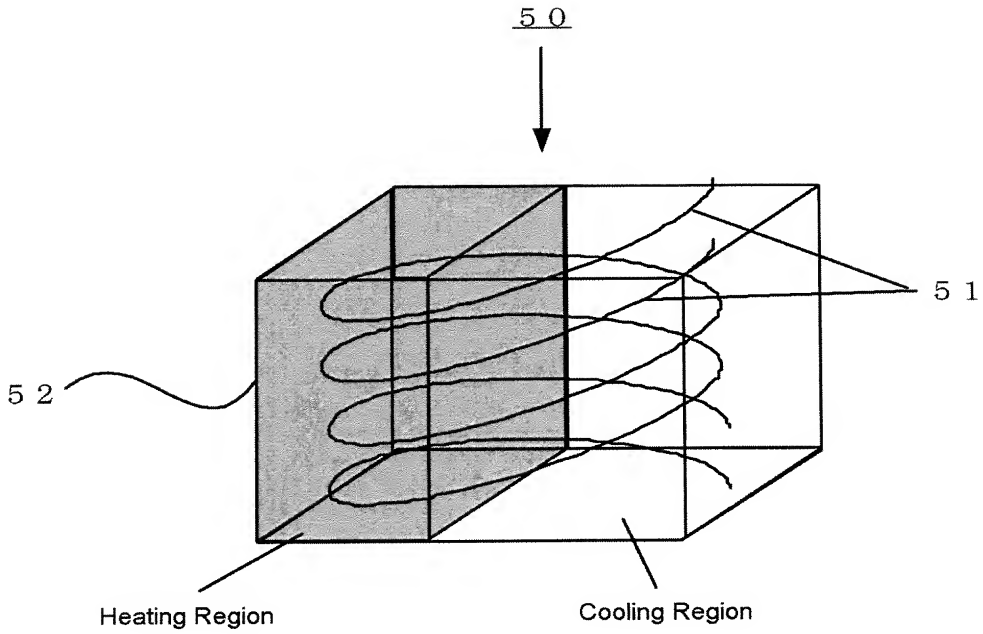
[Fig. 3]


20
1b
Second Reaction Solution
12
1a
1c
Heating Region
1d
11
Recovery or Branching
1e
First Reaction Solution
Cooling Region
2
1f
8
7
13
1g

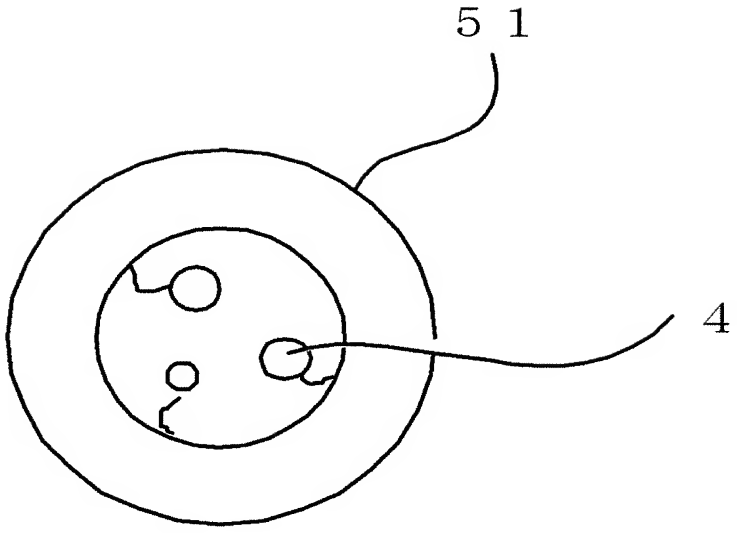
[Fig. 4]


(a)
(b)
1
36
34
31
33
32
38
35
37
1
39
40

[Fig. 5]


50
51
52
Heating Region
Cooling Region

[Fig. 6]



[Type of Document] ABSTRACT

[Summary]

[Problems] It is an object of the present invention to provide a DNA amplifier and a method of DNA amplification performed therewith, by which PCR can be performed in an efficient manner even using a nucleic acid synthetase having no heat resistance property, the nucleic acid synthetase can be recycled, and also the reaction can be scaled up while the isolation and purification of an amplified DNA are facilitated.

[Means of Solving the Problems] After a reaction solution containing at least a nucleic acid to be used as a template, a nucleic acid to be used as a primer, a phosphate compound, and a metal ion is caused to move through one flow channel comprising: a denaturation region in which a denaturation reaction is carried out, the denaturation reaction including denaturing a formed double strand of the nucleic acid to be used as the template; and a regeneration region in which an annealing reaction with a single stranded nucleic acid to be used as the template and the nucleic acid to be used as the primer and a DNA synthesis reaction by a nucleic acid synthetase are carried out, the annealing reaction is carried out while the DNA synthesis is carried out by a nucleic acid synthetase immobilized in the regeneration region.

[Selected Figure] Figure 1